

## Original contribution

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## Time-frequency analysis of Mismatch Negativity (MMN) in healthy Mexican preschool children

Análisis tiempo-frecuencia del Potencial de disparidad (MMN) en niños preescolares mexicanos saludables

### Abstract

**Introduction:** The MMN is an Event-Related Potential frequently studied in auditory change detection tasks. Recent work using time frequency measures has proved promising in understanding the neurophysiology behind auditory change detection. These measures, unfortunately, have not been fully studied in children.

**Objective:** The purpose of this study was to describe the spectro-temporal characteristics of the MMN response using words in healthy Mexicans preschool children.

**Method:** 20 children (10 boys and 10 girls) of preschool age participated a neuropsychological evaluation and an auditory discrimination task during EEG recording. Event Related Spectral Perturbation (ERSP) and Inter-Trial Coherence (ITC) was obtained for frequent and infrequent trials.

**Results:** The ERP showed a typical MMN and LDN response. ERSP increases in the theta band corresponded to the auditory change detection, though no differences between boys and girls was found.

**Conclusions:** In Mexican preschool children, the MMN is like what has been previously reported in adults. However, in contrast to similar studies, sex did not influence the neurophysiological measures. Significance: We established parameters for future research in children using the MMN.

### Keywords

Electroencephalogram, Event-Related Potentials, Event-Related Spectral Perturbations, Inter-Trial Coherence, Audition.

## Resumen

**Introducción:** El MMN es un Potencial Relacionado a Eventos que frecuentemente se estudia ante tareas de detección de cambios auditivos. Trabajos recientes que han utilizado medidas de tiempo-frecuencia han demostrado lo prometedor de comprender la detección neurofisiológica del cambio auditivo, sin embargo, estas medidas no se han estudiado completamente en niños.

**Objetivo:** El propósito de este estudio fue describir las características espectro-temporales de la respuesta MMN utilizando palabras en niños preescolares sanos mexicanos.

**Método:** 20 niños (10 niños y 10 niñas) de edad preescolar participaron en una evaluación neuropsicológica y una tarea de discriminación auditiva durante el registro de EEG. La Perturbación Espectral Relacionada a Eventos (ERSP) y la Coherencia Inter-Ensayos (ITC) se obtuvieron para los estímulos frecuentes e infrecuentes. Resultados: El ERP mostró una respuesta típica de MMN y LDN. El incremento de ERSP en la banda theta correspondió a la detección de cambios auditivos, aunque no se encontraron diferencias entre niños y niñas.

**Conclusiones:** El MMN en niños preescolares mexicanos es similar al que se ha reportado en adultos. Sin embargo, en contraste con estudios similares, el sexo no tuvo efecto sobre las medidas neurofisiológicas. Significancia: Establecimos parámetros para la investigación futura en niños usando el MMN.

### Palabras clave

*Electroencefalograma, Potencial Relacionados a Eventos, Perturbaciones Espectrales Relacionadas a Eventos, Coherencia Inter-Ensayos, Audición.*

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# Introduction

Living organisms need to discriminate between the various characteristics of sound stimuli acquired from the environment to adapt their behavior, to provide answers and to learn. In the case of language, speech perception is associated with the identification and discrimination of the characteristics of auditory stimuli, such as phonemes and words that make up a language.<sup>1</sup>

To perform this auditory discrimination, an adaptation process is produced in which neurons decrease their response to certain stimuli that are presented regularly and give longer responses when the neurons detect a stimulus difference.<sup>2,3</sup>

Neurophysiological techniques such as Event-Related Potentials (ERPs) are useful for analyzing the cortical response to external stimuli. The Mismatch Negativity (MMN) is one of these ERPs and is described as a pre-attentional indicator of deviance detection that reflects a memory process. Each incoming sound is compared with an internal representation of previous stimulation when deviant stimuli are presented within a frequent stimuli sequence.<sup>3,4</sup>

Described for the first time by Näätänen, Gaillard and Mäntysalo,<sup>5</sup> the MMN is a neurophysiological response represented as a negative deflection with certain characteristics: an amplitude of -0.5 to -5  $\mu$ V; with a topographical scalp distribution around frontocentral; spectral power most prominent from 2 to 8.5 Hz;<sup>6</sup> and cortical generators within the primary auditory and prefrontal cortex.<sup>7-10</sup> In addition, deviant stimuli elicit a second negative deflection called the Late Discriminative Negativity (LDN) in children, which decreases with age.<sup>1,11</sup>

In previous studies, auditory phonological processing has been studied using speech stimuli in different languages. MMN responses in healthy children are elicited using complex tones, pseudowords and words. With words, greater amplitudes are found from 150 to 200 ms and 300 to 400 ms.<sup>1</sup> Strotseva-Feinschmidt *et al.*<sup>12</sup> found that the frequent forms

used in German evoked both the MMN and LDN, whereas infrequent forms evoked only the LDN response in 3-year-old children. Ahmadi *et al.*<sup>13</sup> used the MMN to describe the discrimination of phonemes with three different words as deviant stimuli and found that the MMN was about 380-424 ms in healthy children.

Despite these investigations, there is no conclusion about the characteristics of MMN in healthy preschool children using words as stimulus. Ervast *et al.*<sup>14</sup> for example, described the MMN and LDN but using only frequency change in the stimulation. Although the MMN has been described as one of the first cortical correlates of automatic auditory discrimination, current studies have shown that the MMN is not the earliest correlate of deviant stimuli detection in the human auditory cortex and is modulated by earlier ERPs.<sup>3,4,15</sup> The MMN is consistently generated by a set of processes which detect deviant stimuli presentation that occur while auditory processing is performed at multiple levels within the human brain.<sup>3</sup>

The sensory ERPs that appear before the MMN include the exogenous components N1, P1 and N2, which are characterized as obligatory responses of the nervous system to external stimuli. This is supported by the fact that these components of the sensory modality differ in shape and cortical distribution depending on the type of sensory modality in which the stimulus is presented. The N1 component has generators in the dorsal area of the temporal hemispheres and in the superior temporal gyrus of the auditory cortex.<sup>10</sup> The P1 component appears around 50 ms and its cortical generators are in the Heschl gyrus and in the temporal regions of the auditory cortex.<sup>16,17</sup> The N2 component reflects active discrimination by the subject.<sup>18</sup>

The quantification of the MMN and earlier sensory ERPs helps evaluate dynamic changes in the auditory stimulation assessing system of children. This has not only theoretical but also clinical relevance. For example, the absence of a normal MMN in children of typical development could predict the presence of subsequent language disorders.<sup>19</sup> We have also shown that children with language impairment elicit

a MMN with later latencies compared to healthy children.<sup>20</sup>

Besides studying the phase-locked ERPs response, time-frequency analyses have been enjoying a surge in popularity when analyzing EEG. As has been previously stated by Cohen,<sup>21</sup> oscillatory dynamics are more readily associable with their neurophysiological origins compared to ERPs. Time-frequency analyses also allow for the quantification of non-phase locked oscillations which average out in ERPs. This includes event related spectral perturbation (ERSP) and inter-trial phase coherence (ITC).<sup>22</sup> Therefore, time-frequency analyses have shown promise for understanding the neural correlates of behavior and cognition, and complement and extend the findings of ERP-only studies.

Recently, Fujimoto *et al.*<sup>23</sup> studied sex-related differences in gamma oscillation during an auditory oddball task. Using source localization, the authors found that ERSP in females increased in both paracentral regions at 625 ms. Additionally, ERSP in females increased in the medial orbitofrontal regions at 125 ms, and right caudal anterior cingulate cortex regions at 250 ms in both hemispheres, compared to males. Finally, they conclude that these findings indicate a more efficient network architecture in females, corresponding to higher overall cortical connectivity due to greater interhemispheric connectivity in the female brain.

The purpose of this study was to extend previous studies to describe the spectro-temporal characteristics of the MMN response using words in healthy Mexicans preschool children.

## Methods

The present study was prospective, observational, and transversal and was done in the Laboratorio de Psicobiología, Universidad Veracruzana, Xalapa, Veracruz-México.

Thirty-two Mexicans preschool children with an age ranging from 5 to 6.5 years were evaluated.

For the final sample, 20 children were selected (10 males and 10 females matched by age) with mean age of 5.5 months ( $SD \pm 4.6$  months) and paired by age (Appendix 1). The children did not have any diagnosis of developmental disorders and had normal Auditory Attention (AA), Auditory Perception (AP) and Auditory Memory-Coding (AMC), evaluated using the auditory tests included in the Children Neuropsychological Assessment ENI (Evaluación Neuropsicológica Infantil) (Table 1), a neuropsychological test standardized for use in the Mexican population.<sup>24</sup>

The parents or tutors signed a form of informed consent in which the aim of the current study was outlined. Parents were noted that they could suspend any part of the evaluation if they so desired.

## Procedure

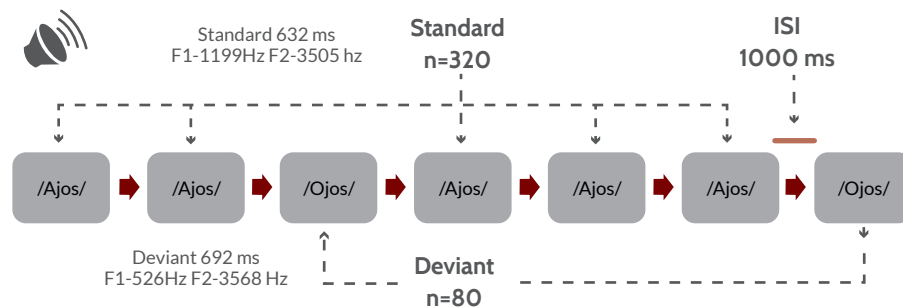
EEG was acquired using a Neuroscan System® with Curry 7.0.10. XS software and a cap with 60 active Ag/AgCl electrodes on the scalp, distributed according to the International System 10-20. This corresponds to the following derivations: FP1-FP2, AF3-AF4, F1-F2, F3-F4, F5-F6, F7-F8, FT7-FT8, FC1-FC2, FC3-FC4, FC5-FC6, C1-C2, C3-C4, C5-C6, T7-T8, TP7-TP8, CP1-CP2, CP3-CP4, CP5-CP6, P1-P2, P3-P4, P5-P6, P7-P8, PO3-PO4, PO5-PO6, PO7-PO8, O1-O2, FPz, Fz, FCz, Cz, CPz, Pz, POz and Oz. An electrode on the left mastoid served as the online reference, the ground electrode was placed in the forehead anterior to FPZ electrode. Four electrodes were used to record vertical and horizontal eye movements (VE1-VE2 and HO1-HO2), and two electrodes located below of collarbone to record cardiac activity (EKG1-EKG2).

A clinical EEG was used to determine if the children had normal electrical brain activity. The MMN was obtained from an auditory passive oddball paradigm (Figure 1), using 320 standard stimuli (/Ajos/) and 80 deviant stimuli (/Ojos/). We selected these stimuli from two word pairs: /Ajos-Ojos/ (Garlic-Eyes) and /Vaso-Paso/ (Glass-Step), since the morphology of the MMN had amplitudes and latencies like that of the reviewed literature.

**Table 1.** Mean ( $\pm$ SD) of the age, quantitative qualification of Auditory Attention (AA), Auditory Perception (AP) and Auditory Memory-Coding (AMC).

Subjects	MEAN ( $\pm$ SD)			
	AGE	AA	AP	AMC
BOYS	68.5 (4.6)	48.7 (35.3)	63.9 (23.1)	52.6 (16.4)
GIRLS	68.1 (4.9)	63.1 (36.7)	75.4 (21.2)	44.4 (24.6)

**Figure 1.** Odd-ball paradigm used to stimulus presentation. Word duration in ms: milliseconds, F1: First formant, F2: Second formant.



The clinical EEG and the MMN were performed in wakefulness with an average duration of 90 minutes. One day before the study, each child went to the registration area to familiarize himself with the place and know what was going to be done, he was told that it would not hurt him and that he should not move. The day of study, the children observed a video without sound so that they could ignore the auditory stimulation. The children were accompanied by their main caregiver, who made sure that the he did not move during the study.<sup>20</sup>

## Data analysis

The EEGLAB toolbox, version 13.5.4b, was used to analyze the data. Recorded data was resampled to 512 Hz, and offline re-referenced to the right mastoid (M2) electrode. Epochs consisted of -200 ms to 500 ms around stimulus onset. Any epoch that contained amplitude of  $\pm 75 \mu V$  was automatically rejected. The FASTICA algorithm was applied to remove eye and heartbeat components.<sup>25</sup> Statistical analyses were done using a mixed factor ANOVA with gender and stimulus type as between

and within factors, respectively. To account for multiple comparisons, we corrected for the False Discovery Rate (FDR) using the corresponding option in EEGLAB 13.5.4b.

For the description of the temporal characteristics of the neurophysiological response of the sample of this research, we defined time-windows to determine the peaks of P1-N2, MMN and LDN. The P1 component was measured around 50 ms, while the N2 component around 150-200 ms.

The MMN was analyzed between 200-300 ms and the LDN between 350-500 ms based on previous studies with preschool children.<sup>1,11,26</sup>

With respect to spectrum characteristics, we analyzed two frequency domain measures: Inter-Trial Coherence (ITC) and the Event-Related Spectral Perturbation (ERSP). The ITC was defined as the degree of synchronization between the epochs (standard and deviant) within the odd-ball paradigm used in the study. The ERSP was the synchronization at specific frequencies over time, starting from the values observed in the baseline.

## Results

### MMN and LDN

A general analysis of the frequent and infrequent stimuli was done at the FCz electrode to observe the P1 and N2 components characteristics (Table 2). The N1 component is not observed.

The electrodes FC3, FCz and FC4 were chosen for MMN and LDN analyses. For a comparison between anterior and posterior areas, O1, Oz and O2 were analyzed.

The MMN was observed at 287.1 ms, with amplitude of  $-0.51 \mu\text{V}$ , and the LDN was observed at 425.8 ms and  $-1.38 \mu\text{V}$  (Figure 2).

In boys, the MMN was found at 282.3 ms with amplitude  $-1.81 \mu\text{V}$  and the LDN was found at 431.2 ms with  $-1.78 \mu\text{V}$ . In girls, the MMN was observed at 288.6 ms with an amplitude of  $0.43 \mu\text{V}$  and the LDN at 395.8 ms with  $-1.26 \mu\text{V}$  (Table 3).

Thus, we found the MMN to have greater amplitude in boys compared to girls, while the LDN showed no differences between the groups (Figure 3).

### ERSP of the MMN

Based on the individual ERSP values of the MMN an average was obtained (Appendix 2). A broadband event related synchronization was observed for frequencies below 20 Hz between 100-200 ms for boys and between 150-300 ms for girls (Figure 4).

While we found a significant difference between boys and girls at the FC4 electrode during early latencies, these differences did not survive correction for multiple comparisons. Theta band synchronization was found in the infrequent stimuli (/Ojos/) for girls only (Figure 5).

This low frequency band synchronization followed a frontocentral distribution in boys and girls (Figure 6).

ITC values showed synchronization for the frequent stimulus presentation between 100 and 200 ms, with frequencies ranging from 3 to 30Hz in both groups (Figure 7).

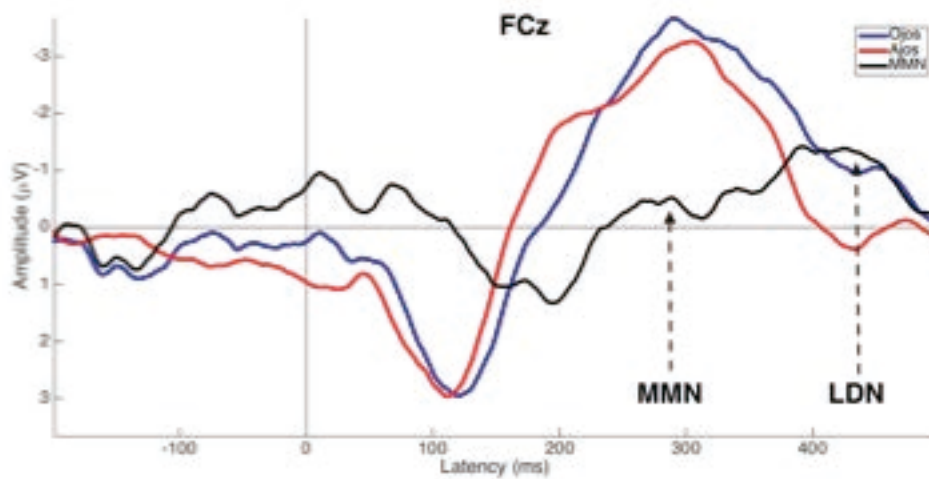
ITC values at Oz showed an increase in synchronization from 100 ms to 200 ms from 5 to 20 Hz for both boys and girls (Figure 8).

Table 2. P1 and N2 components latencies and amplitudes at FCz (n=20).

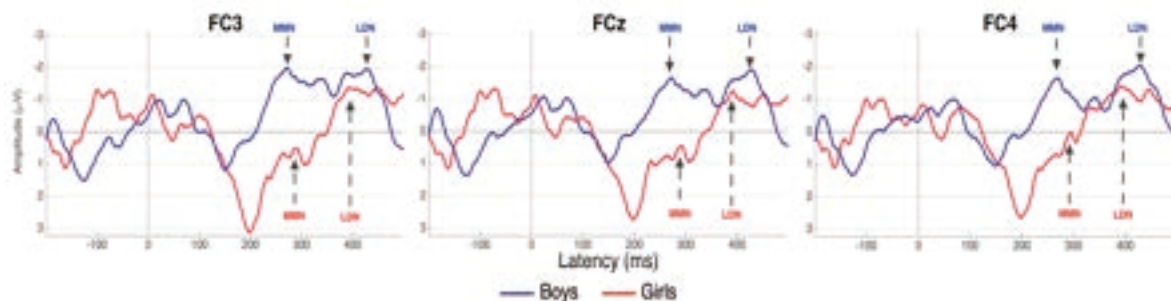
Electrode	Stimuli	P1		N2	
		Latency (ms)	Amplitude ( $\mu\text{V}$ )	Latency (ms)	Amplitude ( $\mu\text{V}$ )
FCZ	/Ajos/	111.3	2.96	304.7	-3.27
	/Ojos/	121.2	2.96	289.1	-3.67

ms = milliseconds  $\mu\text{V}$  = microvolts

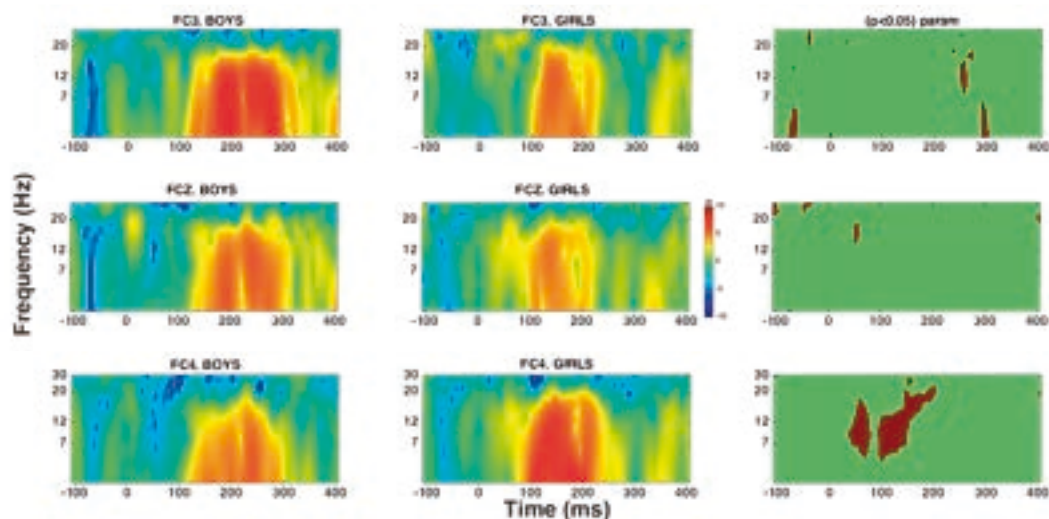


**Figure 2.** Grand average MMN and LDN latencies and amplitudes at FCz.**Table 3.** Average latencies and amplitudes of the MMN y LDN in anterior regions (n=20).

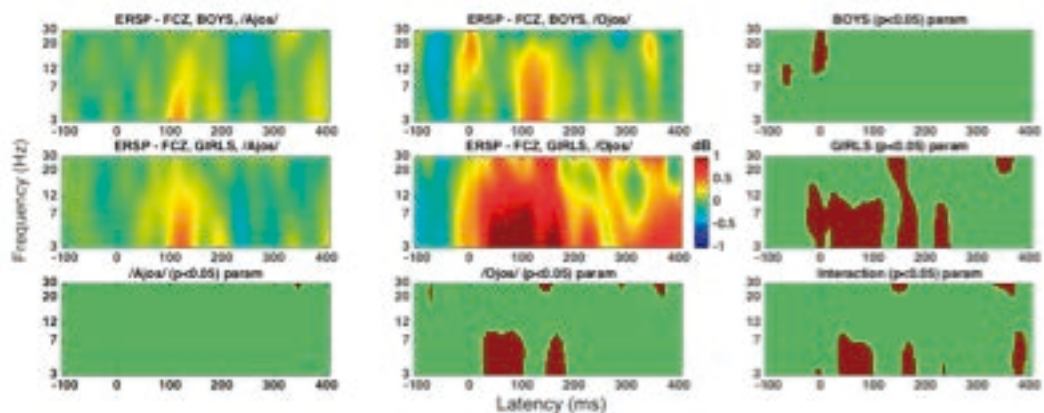
Electrode	Sex	MMN MEAN ( $\pm$ SD)		LDN MEAN ( $\pm$ SD)	
		Latency (ms)	Amplitude ( $\mu$ V)	Latency (ms)	Amplitude ( $\mu$ V)
FC3	Boys	273.2 (38.2)	-2.01 (4.2)	437.3 (37.6)	-2.03 (3.6)
	Girls	288.4 (45.3)	0.52 (1.8)	392.7 (40.7)	-1.96 (3.4)
FCz	Boys	282.3 (40.3)	-1.81 (1.4)	431.2 (22.8)	-1.78 (2.3)
	Girls	288.6 (39.5)	0.43 (3.01)	395.8 (39.9)	-1.26 (2.1)
FC4	Boys	265.4 (48.4)	-1.86 (2.3)	418.8 (52.4)	-2.02 (1.2)
	Girls	293.7 (52.1)	0.03 (3.1)	393.7 (48.3)	-1.43 (1.8)

ms = milliseconds  $\mu$ V = microVolts**Figure 3.** Grand average MMN and LDN at FC3, FCZ and FC4. The blue line corresponds to boys and the red to girls.

**Figure 4.** ERSP of the MMN at FC3, FCz and FC4, of girls (middle) and boys (left). A statistically significant difference ( $p$  not corrected  $< 0.05$ ) was observed at the FC4 electrode.



**Figure 5.** ERSP of /Ajos/ and /Ojos/ at the FCZ.



**Figure 6.** Topographic map of 5 to 20 Hz power for frequent and infrequent stimuli.

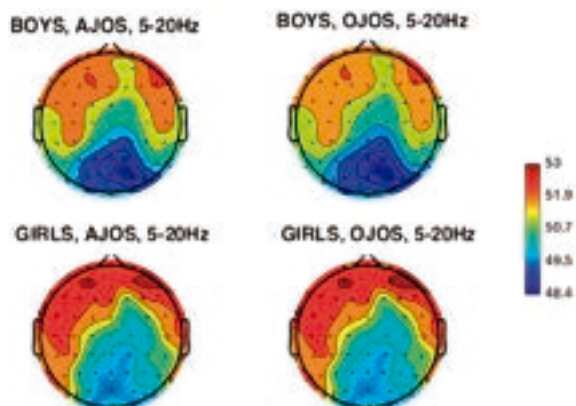




Figure 7. ITC of /Ajos/ and /Ojos/ at the FCz.

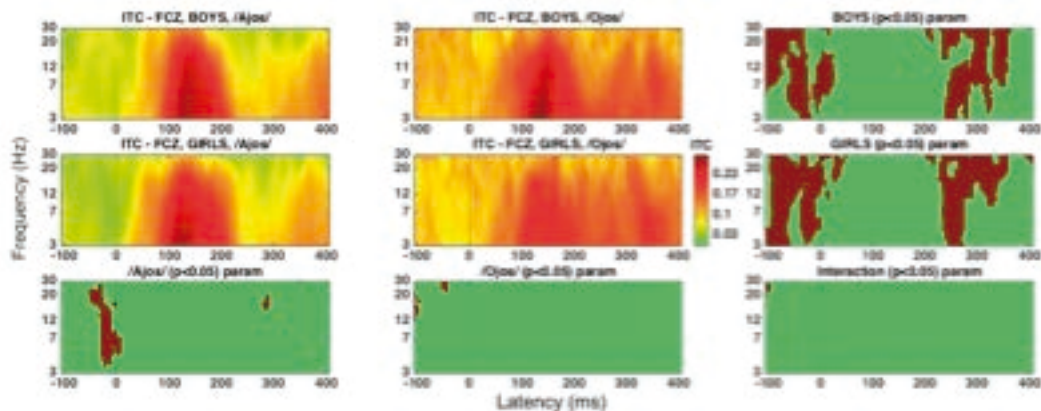
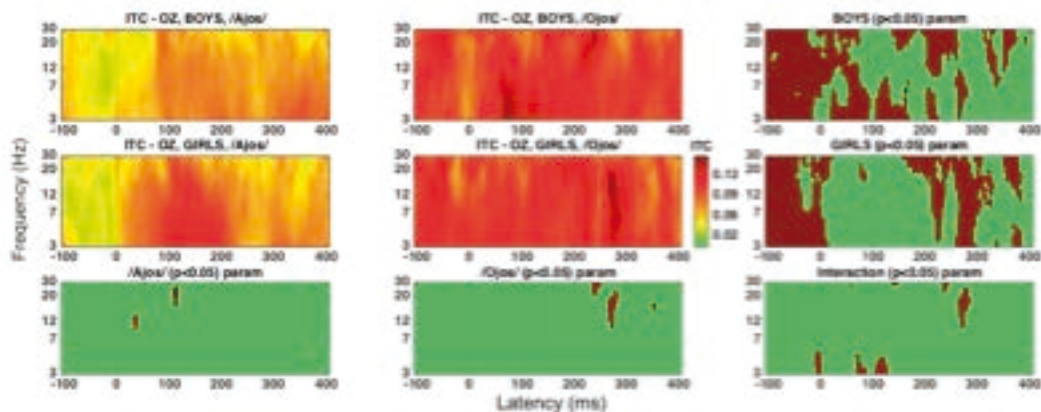


Figure 8. ITC of /Ajos/ and /Ojos/ at the OZ.



## Discussion

Behavioral and neurophysiological studies of development help attend different disorders, as well as adapt teaching methods. In Mexico, it is necessary to have its own behavioral and neurophysiological norms that permit the evaluation of a child's development.

The EEG is a neurophysiological tool that helps discard alterations in brain electrical activity. It also establishes neurophysiological parameters through ERPs for cognitive tasks corresponding to different sensory modalities, so far focused mainly in vision and audition. Within the ERPs, the MMN is a neurophysiological response that has been found is a useful tool to analyze cognitive processes such as

language.<sup>27,28</sup> Taken together, parameters of Mexican children in the preschool stage are necessary, being a relevant stage for the later acquisition of the reading-writing process. This includes the relevance of time-frequency analyses of EEG acquisition with previous studies with children of similar ages.<sup>20</sup> The importance of ITC and ERSP is that these measures define the way different frequency bands of neuronal activity presents with an evoked and induced effect before specific cognitive events.

In children, we observed that although the auditory responses of behavioral tasks of perception, attention, and memory were adequate, between subject differences in language development with relation to the articulation of sounds may be due to perinatal risk factors and to the stimulation received at home or at school. However, the language of 80%

of the children was normal for both boys and girls, echoing several authors who found no differences between genders in language development at 5 years of age. In this way, children should be able to develop a structured language that will continue to develop in expression and comprehension with age.<sup>29</sup>

With regards to the MMN and LDN, we observed responses within expected latencies, amplitude and age distribution and with between-subject differences that were not significant by gender when correcting for multiple comparisons, which leads us to state that, at this stage, the Neurophysiological development among boys and girls was similar.

However, we can say that MMN in boys showed earlier latencies and higher amplitudes than in girls, which is related to better responses of speech language discrimination that has been previously described in cases of typical development.<sup>1</sup> It will be necessary to evaluate how these latencies change throughout the child's school education.

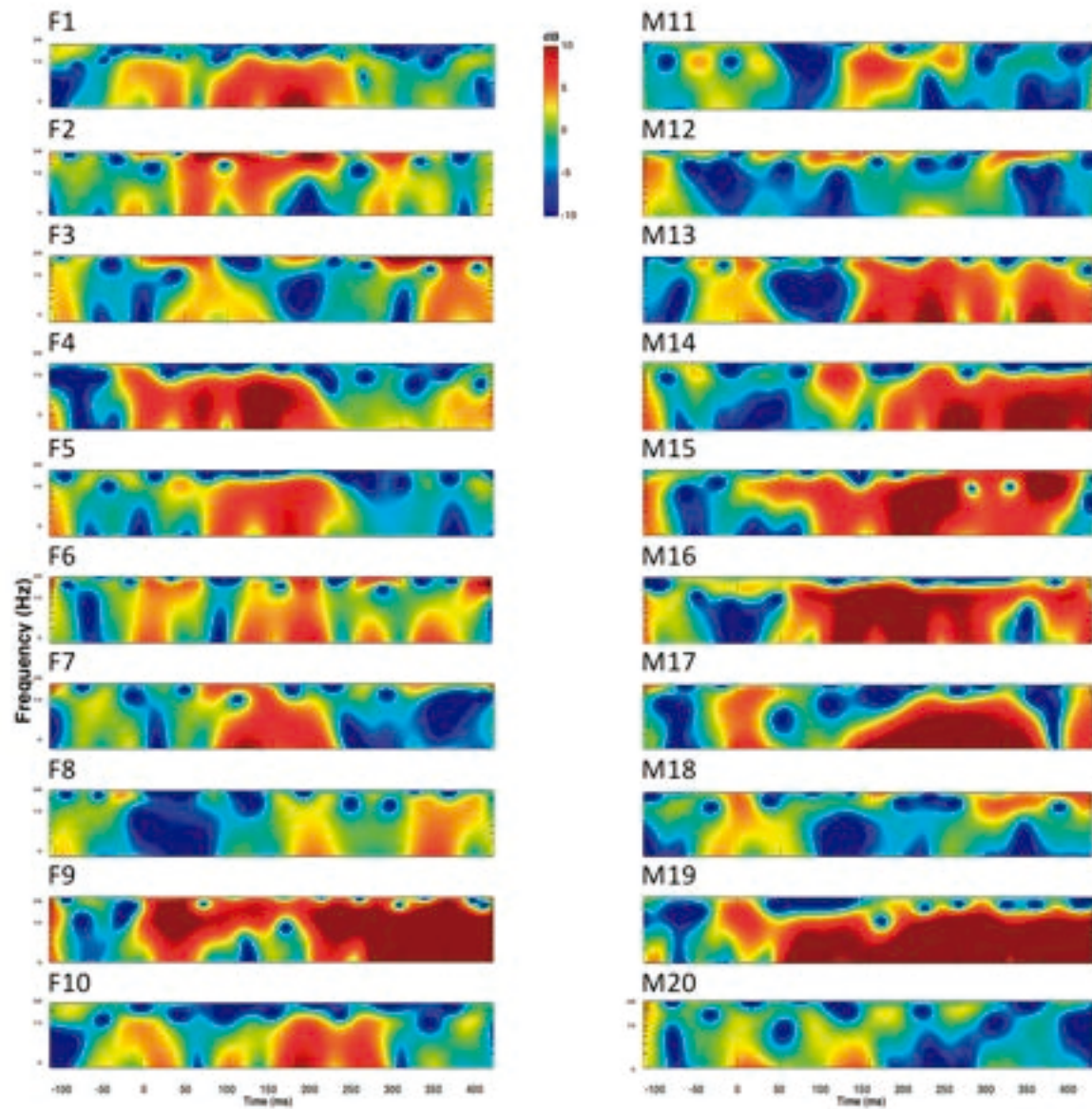
With the time-frequency analysis of event-related potentials we established the methodology to obtain MMN parameters from words previously validated in our laboratory with stimuli selected from a common neuropsychological evaluation for populations of Mexican children. Previous studies have shown data like this study, with an enhanced inter-trial coherence (ITC) in children with dyslexia aged around 6-11 years, but less event-related desynchronization in older children with dyslexia.<sup>30</sup>

As in other studies, we found no significant effect of sex on the theta power. Fujimoto and colleagues<sup>22</sup> found an increase in faster frequencies such as gamma power in males. On the other hand, the MMN has been associated with an increase in theta power for the deviant trials;<sup>31</sup> both results have been described in adults so that it is necessary to continue evaluating children of preschool ages which will allow for any necessary adjustments to have appropriate parameters for our population.

**Appendix 1.** Individual data of age in months, perinatal risk (PR) and articulation in boys and girls.

	Subject	Age in months	PR	Articulation
BOYS	M1	62	Medium	Normal
	M2	62	High	Moderate
	M3	65	High	Normal
	M4	66	Low	Normal
	M5	68	High	Normal
	M6	70	High	Severe
	M7	71	Low	Normal
	M8	72	Medium	Normal
	M9	74	Medium	Mild
	M10	75	Medium	Normal
GIRLS	F1	61	High	Normal
	F2	62	Low	Mild
	F3	65	Low	Normal
	F4	66	Medium	Normal
	F5	65	Medium	Normal
	F6	70	High	Normal
	F7	71	Medium	Normal
	F8	72	Medium	Normal
	F9	74	Low	Normal
	F10	75	High	Normal

**Appendix 2.** Individual Time-frequency analysis of MMN, in the left column the girls and in the right the boys.



## Conclusions

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With the time-frequency analysis of event-related potentials we established for future research, the methodology to obtain MMN parameters from words using the auditory perception tests included in the Children Neuropsychological Assessment ENI standardized for use in the Mexican population.

### Conflicts of interest

None of the authors have potential conflicts of interest to be disclosed.

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